8EHQ-0302-15105

Celanese
CHEMICALS

57442

March 14,2002
DBP-010-02

This submission contains no confidential business information.

Attn: TSCA Section 8(e) Coordinator
Document Processing Center (TS-790)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Contain NO CBI

Dear Sir or Madam:

In accordance with the requirement of TSCA Section 8(e), Celanese, Ltd. hereby submits a final report for a Micronucleus Test in Male and Female NMRI Mice after oral administration of Tributylamine (CAS No. 102-82-9).

A preliminary Acute Oral (gavage) Toxicity Study in mice with tributylamine (CAS# 102-82-9) was conducted to set the dose levels for a Micronucleus Test in mice. Three male and 3 female mice were used at each of the following dose levels: 100, 150, 200 and 500 mg/kg bodyweight. No clinical signs of toxicity or lethality were observed when 3 male and 3 female mice were dosed with 100 mg/kg bodyweight tributylamine. At 150 mg/kg bodyweight, no lethality was observed, but clinical signs possibly indicative of neurotoxicity were observed. These were described as: increased spontaneous activity, uncoordinated gait and ataxic gait. No information was provided regarding incidence, severity or duration of these clinical signs. The dose level was probably near the lethal level, since at the 200 mg/kg bodyweight dose level, 1 female mouse died and at the 500 mg/kg bodyweight dose level 2 male mice and 2 female mice died. A variety of possibly neurotoxic clinical signs were also observed at these 2 higher dose levels.

A variety of clinical signs possibly indicative of neurotoxicity have been reported previously for tributylamine in experimental animals based on the published literature summarized in HSDB (Hazardous Substances Data Bank) and RTECS (Registry of Toxic Effects of Chemical Substances) under the CAS# for tributylamine. These include: restlessness, incoordination, tremors, convulsions or effect on seizure threshold, excitement, somnolence (general depressed activity) and CNS stimulation. Our search of the preceding databases has not identified these effects for tributylamine in the mouse via the oral (gavage) route in the 150 mg/kg bodyweight range.

In the Micronucleus Test itself in addition to positive & negative control mice, 15 male and 15 female mice were dosed with 150 mg/kg bodyweight of tributylamine orally (gavage). No lethality was observed, but clinical signs possibly indicative of neurotoxicity were observed. These were described as: increased spontaneous activity and reduced spontaneous activity. These were transient in nature since 5 hours after dosing all mice were free of these clinical signs. No information was provided on the incidence or severity of these clinical signs. With regard to the mutagenic potential of tributylamine the report prepared by Hoechst concludes that "administration of tributylamine did not lead to a substantial increase of micronucleated polychromatic erythrocytes. It is concluded that tributylamine is not mutagenic in the micronucleus test". Any elevations noted were within the normal range observed in the laboratory according to the report (attached). In this test, the bone marrow of male and female mice was evaluated at 3 time points after dosing (i.e., 24, 48 and 72 hours).

If any further information is required, do not hesitate to contact Debra Phillips, Coordinator, Product Stewardship at 972-443-4703.

Sincerely,

Adrian Becker
Manager, Product Stewardship
Celanese

Enclosure

File: Log No. 5

Bcc: (cover letter only)

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Hoechst

Study Title

Tributylamin

MICRONUCLEUS TEST

IN MALE AND FEMALE NMRI MICE

AFTER ORAL ADMINISTRATION

T01325

Author

Dr. Müller

Study completed on

August 1st, 1989

Performing Laboratory

Pharma Research Toxicology and Pathology
Hoechst Aktiengesellschaft
Postfach 80 03 20
6230 Frankfurt am Main 80

Laboratory Project ID

Study No. 88.1701

Hoechst

Hoechst

STATEMENT OF COMPLIANCE

To the best of my knowledge and belief, this study was conducted in compliance with Good Laboratory Practice regulations. No unforeseen circumstances were observed which might have affected the quality or integrity of the study.

Study Director Dr. Müller

Head of Toxicology Dr. Mayer

Hoechst

Quality Assurance Statement

Hoechst Aktiengesellschaft
Pharma Research
Quality Assurance(GLP)

31.08.1989

Title : Tributylamin
MICRONUCLEUS TEST
IN MALE AND FEMALE NMRI MICE
AFTER ORAL ADMINISTRATION

Date : 17.08.1989

Study No. : 88.1701

This study was periodically inspected and properly signed records of these inspections were submitted to testing facility management and the study director as shown below :

Inspection	Report
21.04.1989	21.04.1989
24.04.1989	24.04.1989
31.08.1989	31.08.1989

Pharma Research
Quality Assurance(GLP)

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CONTENTS

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2. SUMMARY

Tributylamin was tested in the micronucleus test. The test compound was administered orally by gavage to male and female mice. The following doses were tested: 0 and 150 mg **Tributylamin** per kg bodyweight.

The 150 mg per kg bodyweight dose level was chosen since a preliminary study (see appendix p. 23) had shown it to be the maximum non lethal dose.

The animals were treated once with the test compound and according to the test procedure the animals were killed 24, 48 or 72 hours after administration of the test compound.

Endoxan^{R} was used as positiv control substance and was administered orally at a dose of 50 mg per kg bodyweight.

The incidence of micronucleated polychromatic erythrocytes of the animals treated with **Tributylamin** was within the normal range of the negative control. The number of normochromatic erythrocytes containing micronuclei was not increased. The ratio of polychromatic/normochromatic erythrocytes in both male and female animals remained unaffected by the treatment with **Tributylamin** and was statistically not different from the control values.

Endoxan^{R} induced in both males and females a marked statistically significant increase in the number of polychromatic cells with micronuclei, indicating the sensitivity of the system. The ratio of polychromatic erythrocytes to normocytes was not changed to a significant extend.

The results indicate that, under the conditions of the present study, **Tributylamin** is not mutagenic in the micronucleus test.

3. INTRODUCTION

Micronuclei are small secondary nuclear structures resulting from either chromosomal breakage or malfunction of the spindle apparatus of the cells which regulates the distribution of chromosomes during mitosis. The micronucleus test originally described by Schmid (1) is a suitable in vivo method for investigating clastogenic substances and substances which affect the mitotic spindle mechanism. Micronuclei rarely occur in normal dividing cells. The mouse has been chosen for this study since it provides a convenient in vivo mammalian model which has been proposed in the literature (2). The study described was performed according to the OECD guideline No. 474, 1983 (3).

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4. SYNOPSIS

Study No.	:	88.1701
No. of statistical evaluation	:	G5M8905
Test compound	:	**Tributylamin**
Sponsor	:	Ruhrchemie AG, Referat Umweltschutz, Dr. Tihanyi
Test species	:	NMRI mouse
Route of administration	:	oral by gavage
Start of study	:	April 24th, 1989
End of study	:	April 27th, 1989
Doses	:	2 dose groups (0 and 150 mg/kg bwt.) and 1 positive control group
Positive control	:	Endoxan^{R} (50 mg/kg bodyweight p.o.)
Number of animals	:	5 males and 5 females in each dose group
Killing of animals	:	24, 48 or 72 hours after administration (test compound and negative control) 24 h after administration (positive control)

Responsibility

Head of Toxicology	:	Dr. Mayer
Genetic Toxicology	:	Dr. Müller
Statistical evaluation	:	Dr. Rosenkranz
Quality assurance unit (GLP)	:	Ap. Harston
Test facility and archive	:	HOECHST AKTIENGESELLSCHAFT Pharma Research Toxicology and Pathology Post box 80 03 20 6230 Frankfurt 80 Federal Republic of Germany

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5. MATERIAL AND METHODS

5.1 Test compound

Name	:	**Tributylamin**
Code	:	Hoe CG 0113 OA ZD99 0001
CAS No.	:	102-82-9
Chemical nomenclature	:	Tri-n-butylamin
Molecular formula	:	$C_{12}H_{27}N$
Purity	:	99.3 %
Appearance	:	clear up to light yellow fluid
Certificate of analysis	:	04992 from March 8th, 1989
Melting point	:	-70 °C
Boiling point	:	216 - 217 °C
Molecular weight	:	185.35
Specific gravity	:	0.7781
Vapor pressure	:	0.37 hPa
pH - value in water	:	10.6
Batch No.	:	470811
Date of submission	:	December 7th, 1988
Storage conditions	:	dark at 20 °C
Positive control	:	Cyclophosphamid - Endoxan® (Charge 107480)

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5.2 Rational for dose selection

The dose levels for micronucleus testing were selected on the basis of a preliminary study (see appendix, page 23) to determine the acute toxicity and the maximal applicable dose. Oral administration of 200 mg Tributylamin per kg bodyweight has caused partial lethality in male and female mice. The highest sublethal dose of 150 mg/kg bodyweight was selected for the main study.

5.3 Animal species and husbandry

Species	:	NMRI mouse
Strain	:	Hoe: NMRKf (SPF71)
Origin	:	HOECHST AG, Kastengrund, SPF breeding colony
Initial age at test	:	7 weeks
Number of animals	:	70 (35 males / 35 females)
Bodyweight at start of study	:	males : $\bar{x}$= 28.9 g (26 - 34 g)
	:	females: $\bar{x}$= 24.1 g (22 - 28 g)
Acclimatization	:	at least 5 days
Food / water	:	rat/mice diet Altromin 1324 (Altromin-GmbH, Lage/Lippe), ad libitum tap water in plastic bottles, ad libitum
Housing	:	in fully air-conditioned rooms in Macrolon cages (Type 3), on softwood granulate in groups of 5 animals
Room temperature	:	22 ± 2 °C
Relative humidity	:	55 ± 10 %
Lighting time	:	12 hours daily
Animal identification	:	fur-marking with $KMnO_4$ and cage numbering

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5.4 Dose selection and test groups

According to a preliminary study to test the acute toxicity, a dose of 150 mg Tributylamin per kg bodyweight was the maximum non lethal dose level.

Group	Dose (mg/kg bwt.)	Conc.(%) (w/v)	Volume (ml/kg bwt.)	Number of animals and sex	Cage No.	Killing-time (hours p.a.)
1	0	0	10	5 males 5 females	1 2	24
2	150	1.5	10	5 males 5 females	3 4	24
3*	50	0.5	10	5 males 5 females	5 6	24
4	0	0	10	5 males 5 females	13 14	48
5	150	1.5	10	5 males 5 females	7 8	48
6	0	0	10	5 males 5 females	23 24	72
7	150	1.5	10	5 males 5 females	9 10	72

* : Endoxan^{R} (positive control)
hours p.a.: hours after administration

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5.5 Study procedure

5.5.1 Preparation and administration of the test compound

The test compound dilutions were prepared fresh each day. 375 mg Tributylamin were weight in a 25 ml flask, mixed with sesame oil (Oleum Sesami Ph.Eur.III, Fa. Pharm. Fabrik GmbH, Ffm.) and topped up to the calibration mark. A solution was formed.

For the Endoxan^R stock solution, 5 ml distilled water were added to 100 mg Endoxan^R in an injection phial and shaken to form a clear solution. The solutions for administration were prepared from this stock solution. For this purpose, 2 ml of the 2 % stock solution were mixed with 6 ml distilled water.

5.5.2 Preparation of the bone marrow

Extraction of the bone marrow

In conformity with the test procedure the animals were killed by carbon dioxide asphyxiation 24, 48 or 72 hours after application. For each animal, about 3 ml foetal bovine serum was poured into a centrifuge tube. Both femora were removed and the bones freed of muscle tissue. The proximal ends of the femora were opened and the bone marrow flushed into the centrifuge tube. A suspension was formed. The mixture was then centrifuged for 5 minutes at 1200 rpm and almost all the supernatant discarded. One drop of the thoroughly mixed sediment was smeared on a cleaned slide, identified by project code and animal number and air-dried for about 24 hours.

Staining procedure

- 5 minutes in methanol
- 3 minutes in May-Grünwalds solution
- 2 minutes in May-Grünwalds solution diluted 1:1 with distilled water
- brief rinsing twice in distilled water
- 10 minutes staining in 1 part Giemsa solution to 6 parts buffer solution, pH 7.2 (Weise)
- rinsing in distilled water
- drying
- coating with Entellan

5.6 Evaluation

1000 polychromatic erythrocytes were counted for each animal. The number of cells with micronuclei was recorded, not the number of individual micronuclei. As a control measure 1000 mature erythrocytes were also counted and examined for micronuclei. In addition, the ratio of polychromatic to normochromatic erythrocytes was determined. All bone marrow smears for evaluation are coded to ensure that the group to which they belonged remains unknown to the investigator. The number of polychromatic erythrocytes with micronuclei occurring in the 1000 polychromatic erythrocytes counted, and the number of normocytes with micronuclei occurring in the 1000 normocytes counted, were evaluated statistically; comparison of dose groups with the simultaneous control group was performed according to Wilcoxon (paired, one-sided, increase), (4).

The results of the treatment groups (test substance) in the micronucleus test at each dose and killing time were compared with corresponding control values. The ratio of polychromatic to normochromatic erythrocytes was also evaluated statistically by the method of Wilcoxon (paired, two sided) (4). The statistical evaluations were performed using the "Diamant" computer program Version 2.0, supplied by the Department of Information and Communication Hoechst AG. All statistical results are based on a 95 % level of significance. Actual data were also compared with historical controls.

6. RESULTS

Animals were treated with 0 and 150 mg **Tributylamin** per kg bodyweight to study the induction of micronuclei in bone marrow cells of mice.

All animals survived after application of 0 and 150 mg **Tributylamin** per kg bodyweight. The following signs of toxicity were observed: increased spontaneous activity and reduced spontaneous activity.

5 hours after application all animals were free of clinical signs of toxicity.

The bone marrow smears were examined for the occurance of micronuclei in red blood cells. The results are summarized on page 15. Individual data of all animals in all treatment groups are presented on page 16 - 22.

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The incidence of micronucleated polychromatic erythrocytes in the dose groups of **Tributylamin** was within the normal range of the negative control groups. No statistically significant increase of micronucleated polychromatic erythrocytes has been observed. The number of normochromatic erythrocytes with micronuclei did not differ significantly from the values of the simultaneous control animals for each of the three killing times investigated. The ratio of polychromatic erythrocytes to normocytes remained essentially unaffected by the test compound.

Cyclophosphamid (Endoxan^{R}) induced a marked and statistically significant increase of the number of polychromatic erythrocytes with micronuclei in both males and females indicating the sensivity of the test system.

Summarizing it can be stated that, under the conditions described, administration of **Tributylamin** did not lead to a substantial increase of micronucleated polychromatic erythrocytes. It is concluded that **Tributylamin** is not mutagenic in the micronucleus test.

This test was performed according to the methods described. No unforeseen circumstances were observed, which may have affected the quality and integrity of this study. The study was conducted in compliance with the principles of Good Laboratory Practice.

Dr. Mü/Ku

Quality assurance unit

HOECHST AKTIENGESELLSCHAFT
Pharma Research
Toxicology and Pathology

W. Müller 29/8/1989

Dr. Müller
Study director

Dr. Mayer
Head of Toxicology

CHST AG, PHARMA RESEARCH TOXICOLOGY EVALUATION : 08/01/1989

IDY : MICRONUCLEUS TEST PREPARATION : TRIBUTYLAMIN ANIMAL : MOUSE NMRI
ING : SINGLE VEHICLE : SESAME OIL
ITE : ORAL SAMPLING : 24,48,72 HOURS AFTER DOSING
IDY DURATION : 04/24/89-04/27/89 STUDY NO : G5M8905

ATISTICAL METHODS

COMPARISON OF DOSE GROUPS WITH THE SIMULTANEOUS CONTROL GROUP

OPORTION OF POLYCHROMATIC ERYTHROCYTES WITH MICRONUCLEI : WILCOXON (PAIRED,ONE-SIDED,INCREASE)
OPORTION OF NORMOCHROMATIC ERYTHROCYTES WITH MICRONUCLEI : WILCOXON (PAIRED,ONE-SIDED,INCREASE)
TIO OF POLYCHROMATIC TO NORMOCHROMATIC ERYTHROCYTES : WILCOXON (PAIRED,TWO-SIDED)

COMPARISON OF ALL GROUPS WITH NORMAL RANGE (DATED 08/01/89)

COMPARISON OF GROUPS WITH NORMAL RANGES FOR CONTINUOUS PARAMETERS

MBER OF ANIMALS		MICRONUCLEATED ERYTHROCYTES (%)					
OUP NORM.RANGE		POLYCHROMATIC		NORMOCHROMATIC		POLYCHR /NORMOCHR	
#1	#2	M(865)	F(865)	M(865)	F(865)	M(865)	F(865)
5	4	0.40	0.40	0.30	0.30	0.65- 1.32	0.74- 1.43

GROUP OF #1 ANIMALS IS CONSIDERED NORMAL, IF AT LEAST #2 ANIMALS LIE BELOW THE LIMIT
WITHIN THE RANGE SHOWN

E NUMBER IN BRACKETS INDICATES THE RESPECTIVE NUMBER OF CONTROL ANIMALS

L RESULTS ARE BASED ON AN ERROR PROBABILITY OF 5 PER CENT

CHST AG, PHARMA RESEARCH TOXICOLOGY

EVALUATION : 08/01/1989

DY : MICRONUCLEUS TEST
ING : SINGLE
TE : ORAL
DY DURATION : 04/24/89-04/27/89

PREPARATION : TRIBUTYLAMIN
VEHICLE : SESAME OIL
SAMPLING : 24,48,72 HOURS AFTER DOSING
STUDY NO : G5M8905

ANIMAL : MOUSE NMRI
REPORT NR : 89.1015

MARY OF FINDINGS IN BONE MARROW ERYTHROCYTES

.	DOSE MG/KG	SAMPL. AFT. DOSING	NUMBER OF ANMLS	ERYTHROCYTES POLY MEAN	ERYTHROCYTES NORMO MEAN	P/N MEAN	P/N SD		ERYTHROCYTES WITH MICRONUCLEI POLY NO	POLY %	POLY (MEAN) SD		POLY MUT.I.	ERYTHROCYTES WITH MICRONUCLEI NORMO (MEAN) NO	NORMO %	NORMO (MEAN) SD		NORMO MUT.I.
E	CONTROL	24H	5	1000	1000	1.03	0.17	I	3	0.28	0.13	I	1.0	1	0.06	0.05	I	1.0
	150	24H	5	1000	1000	1.10	0.09	-I	3	0.28	0.15	-I	1.0	2	0.20	0.10	*I	3.3
	P.CONT.	24H	5	1000	1000	0.86	0.08	-I	25	2.52	0.64	*A	9.0	1	0.10	0.10	-I	1.7
ALE	CONTROL	24H	5	1000	1000	0.98	0.17	I	2	0.22	0.13	I	1.0	2	0.16	0.15	I	1.0
	150	24H	5	1000	1000	0.98	0.19	-I	2	0.16	0.15	-I	0.7	1	0.12	0.11	-I	0.8
	P.CONT.	24H	5	1000	1000	0.81	0.11	-I	29	2.92	0.48	*A	13.3	2	0.20	0.07	-I	1.3
E	CONTROL	48H	5	1000	1000	1.08	0.28	A	2	0.24	0.15	I	1.0	1	0.10	0.14	I	1.0
	150	48H	5	1000	1000	0.93	0.20	-(I)	2	0.24	0.11	-I	1.0	2	0.16	0.09	-I	1.6
ALE	CONTROL	48H	5	1000	1000	1.25	0.16	I	2	0.22	0.16	I	1.0	2	0.16	0.15	I	1.0
	150	48H	5	1000	1000	0.99	0.15	-I	3	0.28	0.13	-I	1.3	1	0.12	0.04	-I	0.8
E	CONTROL	72H	5	1000	1000	1.01	0.28	I	0	0.02	0.04	I	1.0	0	0.00	0.00	I	-
	150	72H	5	1000	1000	0.97	0.18	-I	1	0.14	0.13	-I	7.0	0	0.00	0.00	-I	-
ALE	CONTROL	72H	5	1000	1000	1.10	0.07	I	1	0.08	0.13	I	1.0	0	0.00	0.00	I	-
	150	72H	5	1000	1000	0.90	0.07	*I	1	0.08	0.08	-I	1.0	1	0.08	0.04	*I	-

.I.=MUTAGENIC INDEX=ERY WITH MICRONUCLEI IN DOSE GROUP /ERY WITH MICRONUCLEI IN CONTROL
TROL MG/KG KGW.
ONT. = POSITIVE CONTROL = ENDOXAN (50 MG/KG KGW.,PO)

ULTS IN BRACKETS SINCE CONTROL LIES OUTSIDE NORMAL RANGE
NO DIFFERENCE FROM CONTROL (P>.05)
WITHIN THE NORMAL RANGE

NT= CONTROL NOT TREATED
* = SIGNIFICANTLY DIFFERENT FROM CONTROL (P<.05)
A = OUTSIDE THE NORMAL RANGE

CHST AG, PHARMA RESEARCH TOXICOLOGY

EVALUATION : 08/01/1989

DY : MICRONUCLEUS TEST
ING : SINGLE
TE : ORAL
DY DURATION : 04/24/89-04/27/89

PREPARATION : TRIBUTYLAMIN
VEHICLE : SESAME OIL
SAMPLING : 24 HOURS AFTER DOSING
STUDY NO : G5M8905

ANIMAL : MOUSE NMRI

DINGS IN BONE MARROW ERYTHROCYTES

DOSE: CONTROL MG/KG KGW.

POLYCHROMATIC ERYTHROCYTES

N = NORMOCHROMATIC ERYTHROCYTES

MALE

IL	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P	%	N	%
1	32	1000	1000	1.11	4	0.40	1	0.10
2	31	1000	1000	0.85	4	0.40	1	0.10
3	29	1000	1000	1.16	3	0.30	1	0.10
4	31	1000	1000	0.83	1	0.10	0	0.00
5	29	1000	1000	1.18	2	0.20	0	0.00
	5							
AN	30	1000	1000	1.03	3	0.28	1	0.06
	1	0	0	0.17	1	0.13	1	0.05
T.I.						1.0		1.0

FEMALE

ANML NO /89	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P	%	N	%
6	23	1000	1000	1.21	3	0.30	2	0.20
7	25	1000	1000	1.07	1	0.10	0	0.00
8	23	1000	1000	0.94	1	0.10	1	0.10
9	25	1000	1000	0.92	4	0.40	4	0.40
10	24	1000	1000	0.76	2	0.20	1	0.10
N	5							
MEAN	24	1000	1000	0.98	2	0.22	2	0.16
SD	1	0	0	0.17	1	0.13	2	0.15
MUT.I.						1.0		1.0

T.I.=MUTAGENIC INDEX=ERY WITH MICRONUCLEI IN DOSE GROUP /ERY WITH MICRONUCLEI IN CONTROL

HST AG, PHARMA RESEARCH TOXICOLOGY

EVALUATION : 08/01/1989

Y : MICRONUCLEUS TEST
NG : SINGLE
E : ORAL
Y DURATION : 04/24/89-04/27/89

PREPARATION : TRIBUTYLAMIN
VEHICLE : SESAME OIL
SAMPLING : 24 HOURS AFTER DOSING
STUDY NO : G5M8905

ANIMAL : MOUSE NMRI

INGS IN BONE MARROW ERYTHROCYTES

DOSE: 150 MG/KG KGW.

POLYCHROMATIC ERYTHROCYTES

N = NORMOCHROMATIC ERYTHROCYTES

MALE								FEMALE							
	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P	%	N	%	ANML NO /89	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P	%	N	%
	30	1000	1000	1.08	5	0.50	1	0.10	16	24	1000	1000	1.17	0	0.00	3	0.30
	29	1000	1000	1.16	3	0.30	3	0.30	17	26	1000	1000	1.00	2	0.20	1	0.10
	28	1000	1000	0.97	1	0.10	1	0.10	18	26	1000	1000	1.16	4	0.40	1	0.10
	30	1000	1000	1.19	3	0.30	2	0.20	19	28	1000	1000	0.72	1	0.10	1	0.10
	30	1000	1000	1.09	2	0.20	3	0.30	20	23	1000	1000	0.85	1	0.10	0	0.00
	5								N	5							
	29	1000	1000	1.10	3	0.28	2	0.20	MEAN	25	1000	1000	0.98	2	0.16	1	0.12
	1	0	0	0.09	1	0.15	1	0.10	SD	2	0	0	0.19	2	0.15	1	0.11
.I.						1.0		3.3	MUT.I.						0.7		0.8

.I.=MUTAGENIC INDEX=ERY WITH MICRONUCLEI IN DOSE GROUP /ERY WITH MICRONUCLEI IN CONTROL

CHST AG, PHARMA RESEARCH TOXICOLOGY EVALUATION : 08/01/1989

DY : MICRONUCLEUS TEST PREPARATION : TRIBUTYLAMIN ANIMAL : MOUSE NMRI
ING : SINGLE VEHICLE : SESAME OIL
TE : ORAL SAMPLING : 24 HOURS AFTER DOSING
DY DURATION : 04/24/89-04/27/89 STUDY NO : G5M8905

DINGS IN BONE MARROW ERYTHROCYTES DOSE: P.CONT. 50 MG/KG KGW. ENDOXAN

POLYCHROMATIC ERYTHROCYTES N = NORMOCHROMATIC ERYTHROCYTES

	MALE							FEMALE						
IL	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P	%	N	%	ANML NO /89	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P	%	N	%
1	33	1000	1000	0.82	19	1.90	0	0.00	26	25	1000	1000	0.76	32	3.20	2	0.20
2	28	1000	1000	0.92	26	2.60	2	0.20	27	25	1000	1000	1.00	31	3.10	3	0.30
3	29	1000	1000	0.90	28	2.80	2	0.20	28	24	1000	1000	0.79	21	2.10	1	0.10
4	27	1000	1000	0.92	19	1.90	0	0.00	29	22	1000	1000	0.77	29	2.90	2	0.20
5	28	1000	1000	0.75	34	3.40	1	0.10	30	24	1000	1000	0.72	33	3.30	2	0.20
	5								N	5							
N	29	1000	1000	0.86	25	2.52	1	0.10	MEAN	24	1000	1000	0.81	29	2.92	2	0.20
	2	0	0	0.08	6	0.64	1	0.10	SD	1	0	0	0.11	5	0.48	1	0.07
.I.						9.0		1.7	MUT.I.						13.3		1.3

.I.=MUTAGENIC INDEX=ERY WITH MICRONUCLEI IN DOSE GROUP /ERY WITH MICRONUCLEI IN CONTROL

:HST AG, PHARMA RESEARCH TOXICOLOGY

EVALUATION : 08/01/1989

)Y : MICRONUCLEUS TEST
:NG : SINGLE
:E : ORAL
)Y DURATION : 04/24/89-04/27/89

PREPARATION : TRIBUTYLAMIN
VEHICLE : SESAME OIL
SAMPLING : 48 HOURS AFTER DOSING
STUDY NO : G5M8905

ANIMAL : MOUSE NMRI

)INGS IN BONE MARROW ERYTHROCYTES

DOSE: CONTROL MG/KG KGW.

POLYCHROMATIC ERYTHROCYTES

N = NORMOCHROMATIC ERYTHROCYTES

	MALE							FEMALE						
	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P %	N %	ANML NO /89	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P %	N %
1	31	1000	1000	1.36	5 0.50	0 0.00	66	23	1000	1000	1.08	0 0.00	0 0.00
2	29	1000	1000	0.79	2 0.20	0 0.00	67	24	1000	1000	1.38	3 0.30	3 0.30
3	31	1000	1000	0.79	2 0.20	0 0.00	68	24	1000	1000	1.24	1 0.10	3 0.30
4	31	1000	1000	1.36	1 0.10	2 0.20	69	23	1000	1000	1.12	4 0.40	2 0.20
5	30	1000	1000	1.11	2 0.20	3 0.30	70	25	1000	1000	1.44	3 0.30	0 0.00
	5						N	5					
N	30	1000	1000	1.08	2 0.24	1 0.10	MEAN	24	1000	1000	1.25	2 0.22	2 0.16
	1	0	0	0.28	2 0.15	1 0.14	SD	1	0	0	0.16	2 0.16	2 0.15
.I.					1.0	1.0	MUT.I.					1.0	1.0

.I.=MUTAGENIC INDEX=ERY WITH MICRONUCLEI IN DOSE GROUP /ERY WITH MICRONUCLEI IN CONTROL

CHST AG, PHARMA RESEARCH TOXICOLOGY EVALUATION : 08/01/1989

IDY : MICRONUCLEUS TEST PREPARATION : TRIBUTYLAMIN ANIMAL : MOUSE NMRI
ING : SINGLE VEHICLE : SESAME OIL
ITE : ORAL SAMPLING : 48 HOURS AFTER DOSING
IDY DURATION : 04/24/89-04/27/89 STUDY NO : G5M8905

IDINGS IN BONE MARROW ERYTHROCYTES DOSE: 150 MG/KG KGW.

= POLYCHROMATIC ERYTHROCYTES N = NORMOCHROMATIC ERYTHROCYTES

MALE

IL	BW G	ERYTHROCYTES			ERYTHROCYTES WITH MICRONUCLEI			
)		P	N	P/N	P	%	N	%
31	31	1000	1000	0.70	2	0.20	2	0.20
32	29	1000	1000	1.09	2	0.20	1	0.10
33	29	1000	1000	0.71	1	0.10	1	0.10
34	30	1000	1000	1.07	4	0.40	1	0.10
35	30	1000	1000	1.06	3	0.30	3	0.30
	5							
AN	30	1000	1000	0.93	2	0.24	2	0.16
	1	0	0	0.20	1	0.11	1	0.09
T.I.						1.0		1.6

FEMALE

ANML NO /89	BW G	ERYTHROCYTES			ERYTHROCYTES WITH MICRONUCLEI			
		P	N	P/N	P	%	N	%
36	23	1000	1000	1.10	5	0.50	1	0.10
37	25	1000	1000	1.18	2	0.20	1	0.10
38	27	1000	1000	0.82	2	0.20	1	0.10
39	23	1000	1000	0.93	2	0.20	1	0.10
40	27	1000	1000	0.92	3	0.30	2	0.20
N	5							
MEAN	25	1000	1000	0.99	3	0.28	1	0.12
SD	2	0	0	0.15	1	0.13	0	0.04
MUT.I.						1.3		0.8

T.I.=MUTAGENIC INDEX=ERY WITH MICRONUCLEI IN DOSE GROUP /ERY WITH MICRONUCLEI IN CONTROL

HST AG, PHARMA RESEARCH TOXICOLOGY

EVALUATION : 08/01/1989

Y : MICRONUCLEUS TEST
NG : SINGLE
E : ORAL
Y DURATION : 04/24/89-04/27/89

PREPARATION : TRIBUTYLAMIN
VEHICLE : SESAME OIL
SAMPLING : 72 HOURS AFTER DOSING
STUDY NO : G5M8905

ANIMAL : MOUSE NMRI

INGS IN BONE MARROW ERYTHROCYTES

DOSE: CONTROL MG/KG KGW.

POLYCHROMATIC ERYTHROCYTES

N = NORMOCHROMATIC ERYTHROCYTES

MALE								FEMALE							
	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P	%	N	%	ANML NO /89	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P	%	N	%
	34	1000	1000	1.15	0	0.00	0	0.00	116	23	1000	1000	1.05	0	0.00	0	0.00
	32	1000	1000	0.81	0	0.00	0	0.00	117	23	1000	1000	1.13	3	0.30	0	0.00
	30	1000	1000	1.43	0	0.00	0	0.00	118	25	1000	1000	1.10	0	0.00	0	0.00
	31	1000	1000	0.72	1	0.10	0	0.00	119	26	1000	1000	1.21	0	0.00	0	0.00
	31	1000	1000	0.95	0	0.00	0	0.00	120	22	1000	1000	1.02	1	0.10	0	0.00
	5								N	5							
	32	1000	1000	1.01	0	0.02	0	0.00	MEAN	24	1000	1000	1.10	1	0.08	0	0.00
	2	0	0	0.28	0	0.04	0	0.00	SD	2	0	0	0.07	1	0.13	0	0.00
.I.						1.0		-	MUT.I.						1.0		-

.I.=MUTAGENIC INDEX=ERY WITH MICRONUCLEI IN DOSE GROUP /ERY WITH MICRONUCLEI IN CONTROL

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EVALUATION : 08/01/1989

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ING : SINGLE
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DY DURATION : 04/24/89-04/27/89

PREPARATION : TRIBUTYLAMIN
VEHICLE : SESAME OIL
SAMPLING : 72 HOURS AFTER DOSING
STUDY NO : G5M8905

ANIMAL : MOUSE NMRI

DINGS IN BONE MARROW ERYTHROCYTES

DOSE: 150 MG/KG KGW.

POLYCHROMATIC ERYTHROCYTES

N = NORMOCHROMATIC ERYTHROCYTES

	MALE							FEMALE						
IL /)	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P	%	N	%	ANML NO /89	BW G	ERYTHROCYTES P	N	P/N	ERYTHROCYTES WITH MICRONUCLEI P	%	N	%
1	26	1000	1000	0.84	0	0.00	0	0.00	46	25	1000	1000	0.89	0	0.00	1	0.10
2	28	1000	1000	0.93	3	0.30	0	0.00	47	23	1000	1000	0.80	1	0.10	0	0.00
3	27	1000	1000	1.21	2	0.20	0	0.00	48	22	1000	1000	0.89	2	0.20	1	0.10
4	27	1000	1000	0.79	0	0.00	0	0.00	49	22	1000	1000	0.93	0	0.00	1	0.10
5	28	1000	1000	1.10	2	0.20	0	0.00	50	22	1000	1000	0.99	1	0.10	1	0.10
	5								N	5							
AN	27	1000	1000	0.97	1	0.14	0	0.00	MEAN	23	1000	1000	0.90	1	0.08	1	0.08
	1	0	0	0.18	1	0.13	0	0.00	SD	1	0	0	0.07	1	0.08	0	0.04
T.I.						7.0		-	MUT.I.						1.0		-

T.I.=MUTAGENIC INDEX=ERY WITH MICRONUCLEI IN DOSE GROUP /ERY WITH MICRONUCLEI IN CONTROL

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8. APPENDIX

8.1 Preliminary study

Preliminary studies were conducted to determine the highest administrable non lethal dose level.

Preliminary study	: January 31st, 1989 - February 22nd, 1989
1st dose	: 100 mg/kg bodyweight Tributylamin
Number of animals used	: 3 males and 3 females
Clinical signs	: no signs of toxicity
Lethality rate	: 0 out of 3 males 0 out of 3 females
2nd dose	: 500 mg/kg bodyweight Tributylamin
Number of animals used	: 3 males and 3 females
Clinical signs	: widened palpebral fissures, saltatory and rolling convulsions, irregular breathing, uncoordinated gait, " Straub " tail and increased spontaneous activity
Lethality rate	: 2 out of 3 males 2 out of 3 females
3rd dose	: 200 mg/kg bodyweight Tributylamin
Number of animals used	: 3 males and 3 females
Clinical signs	: increased spontaneous activity, uncoordinated gait, excitement, widened palpebral fissures, narrowed palpebral fissures, reduced spontaneous activity, irregular breathing, gasping respiration, clonic convulsions and " Straub " tail
Lethality rate	: 0 out of 3 males 1 out of 3 females
4th dose	: 150 mg/kg bodyweight Tributylamin
Number of animals used	: 3 males and 3 females
Clinical signs	: increased spontaneous activity, uncoordinated gait and ataxic gait
Lethality rate	: 0 out of 3 males 0 out of 3 females

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9. REFERENCES

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5. Wilcoxon, M. Hollander and D.A. Wolfe, Nonparametric Statistical Methods, Wiley, New York, 1973

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